BMP MOSQUITO PRODUCTION

STUDY PLAN

CALIFORNIA DEPARTMENT OF HEALTH SERVICES

VECTOR-BORNE DISEASE SECTION

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Overview and Objectives

The California Department of Transportation (Caltrans) is currently completing development of the Stormwater Best Management Practice (BMP) Retrofit Pilot Program in Los Angeles (Caltrans District 7) and San Diego (District 11) counties. The primary objectives of this project are to determine the pollutant removal efficiency of the BMPs, assess their operational and maintenance requirements, and monitor and abate mosquitoes that pose a potential threat to public health. The purpose of this report is to outline the experimental design for determining which BMP designs are least conducive for mosquito production. This study will involve sampling of mosquito larvae and pupae from 34 BMPs located in Los Angeles and San Diego counties for a two-year period beginning in the summer of 1999. This study will involve several collaborating agencies. Local vector control districts that have jurisdiction in the region where the BMPs are located will conduct sampling of immature mosquitoes. The role of the California Department of Health Services/Vector Borne Disease Section (DHS/VBDS) is to coordinate the efforts of the vector control districts and incorporate all the data generated into a database. DHS/VBDS will also directly monitor biological, physical, and chemical parameters such as vegetation type and density, predator occurrence, and water quality for correlation with mosquito population estimates.

Rationale and Introduction

The climate in southern California is conducive to mosquito production and pathogen transmission. Abatement of mosquitoes is of importance to reduce their nuisance and transmission of disease-causing pathogens (e.g., encephalitis virus, dog heartworm, and malaria) to humans and animals. The increase in urban population and the addition of new sources of standing water in southern California creates a potential for proliferation of pestiferous mosquitoes and vector-borne diseases. Every year encephalitis viruses are detected in sentinel bird flocks used in the early warning system of virus activity within California (Reeves 1990). Construction of BMPs for stormwater runoff in such an environment will provide additional habitat for mosquitoes that utilize storm drains, organically rich ponds, and wetlands, thereby enhancing the potential for disease outbreaks.

Although the BMPs are artificial structures, they will undergo natural successive changes and evolve into ecosystems that closely resemble ephemeral aquatic habitats that are characterized by cycles of drying and subsequent inundation (Souza 1984). Such an ecosystem is susceptible to disturbance from stormwater flash floods and rapid drying that result in adverse effects on aquatic populations. These adverse effects impact aquatic populations by directly causing mortality or indirectly by reducing available resources, and by increasing competitive or predatory interactions (Aspbury and Juliano 1998). To understand the complex interrelated dynamics of such an aquatic ecosystem it is necessary to monitor long-term successive changes. As a preliminary attempt to understand what interactions are expected in these ephemeral habitats, a two-year project is planned to survey the colonization and utilization of these habitats by vector species.

Since mosquitoes are pestiferous and capable of transmitting diseases of major public health importance, establishment of BMPs in urban environments for the purposes of reducing stormwater pollutants may present a substantial health risk. If these BMPs are to be adopted as a primary method of cleaning stormwater runoff, and to be built throughout the state and the nation, then the potential of health risk must be considered in the decision making process. The project objectives are to study, document, and report on the capacity of these devices to support breeding populations of mosquitoes, and to recommend procedures that may be taken to reduce this undesirable outcome. In addition to these responsibilities, it is pertinent to such an effort to

document the interaction of mosquitoes and other organisms within these artificial ecosystems. The procedures and methods to achieve these goals are outlined below.

Methods and Procedures

This project will consist of monitoring immature stages of mosquitoes and other nuisance insects. When possible, monitoring of mosquito larvae will be conducted by standard techniques. However, the habitats provided by these BMPs are diverse and will require significant effort to determine which sampling technique best provides a comparable data set that reflects mosquito abundance. Decisions to implement mosquito control will be based on the abundance of larval and pupal stages within the BMPs.

Sampling of immature mosquitoes

When possible, larval mosquitoes will be sampled by standard dipping techniques (Service 1993). The choice of sampling technique depends on the mosquito species likely to be present, habitat type, and weather conditions. The shallow and irregular surfaces found in some of the BMPs will present further challenges in sampling mosquito larvae. For instance, in shallow water with a depth less than the height of the dipper's cup, the dipper should be held at a 45° angle and the water allowed to flow into the dipper (O'Malley 1995). Such samples will be recorded in a standard dipper equivalent. Some BMP types present special sampling challenges. For instance, the multi-chambered treatment train (MCTT) BMPs have a series of sedimentation tubes, creating an unconventional mosquito habitat and a challenge for larval sampling. The use of emergence traps, dip-nets, and perhaps other novel devices, will be necessary in some instances. Some vector control districts have already made attempts at devising sampling techniques for some BMP types. The "Kluh" dipper seems useful in sampling the outlet ditch in the MCTT but not for the chambers themselves (S. Kluh, Personal Comm). Kluh's dipper is a standard dipper cup without a handle. The cup is weighted on one side with attached strings used to collect a sample in the MCTT.

At each BMP site, the presence or absence of water will be recorded. Where standing water is present, the location, the approximate surface area, and the depth shall be noted. Three to five (3-5) dipper samples (or equivalent) will be taken around the perimeter of the standing water within the BMP. If the amount of water present is too small to allow for a dipper sample,

the use of novel devices such as "turkey basters" or aquatic pipettes (BioQuip Products, Inc., Gardena, CA) may be required; and these samples will be recorded in equivalent units of larvae per dip. The larval and pupal samples will be preserved in 80% ethanol.

Mosquito larvae in steep-sided chambers such as MCTT, media filters, or catch basins will be sampled by a "Kluh" dipper (S. Kluh, Personal Comm), dip-net, or by strainers attached to lines (Service 1993). A representative sample will be taken and the data transformed into larvae per dip equivalents. Any material caught on the net will be rinsed into a labeled sample jar or vial and then preserved in 80% ethanol.

Immature mosquito populations will be monitored at two to four week intervals. Samples will be transported to the laboratory, then removed from the preserving media, placed in gridded-petri dishes and identified using the Bohart and Washino (1978) manual. Samples will be counted and recorded according to their stages (larvae stage I-IV, Pupae); those larvae and pupae that cannot be identified to species level will be recorded at the genus level.

Mosquito Predator Monitoring

As BMP habitats become more stable systems, various mosquito predators may become established, forming a sustainable relationship with their mosquito and other aquatic invertebrate prey. The presence of predatory species in these BMPs does provide some control of pestiferous mosquitoes, and above all, serves as an important indicator of ecological stability. In less than ideal conditions, where BMPs may lack consistent treatment and maintenance, mosquito control may depend solely on predatory species at these habitats. The monitoring of mosquito predators at the BMP study sites provides pertinent and important data that may reflect such conditions.

The treatment of BMPs with Altosid® against mosquito breeding does not significantly affect larval stages of mosquitoes or non-target organisms such as mosquito predators. This allows for a stable larval population of mosquitoes that will eventually sustain a dynamic population of predator species that will be correlated to mosquito production.

Although the temporary nature of some of the BMPs may not allow stability to occur, natural and artificially introduced predators, such as mosquito fish, will be monitored at all sites every two to four weeks. Several sampling techniques may be necessary to collect information that can be correlated to mosquito abundance. Minnow traps and dip-nets will be used to sample

mosquito fish and invertebrate predators such as hydrophilids, notonectids, belostomatids, and others. The samples collected will be preserved, identified, and recorded into the database.

Vegetation Sampling

A general description of plants will be employed, classifying plants as emergent or submergent flora. Algae, which are categorized as submergent plants, will be estimated by sampling and will be identified to order or family level. This and other submergent vegetation will also be sampled and identified.

Emergent vegetation in a body of standing water positively impacts larval mosquito abundance (Bailey and Gieke 1968) by providing larvae with refuge from predation and physical disturbances, and by making food resources available for mosquito larvae (Collins and Resh 1989). Standing water that contains vegetation is a particularly important habitat characteristic for several species of mosquitoes. Unlike the concrete-lined BMPs (i.e., media filters and extended detention basins), the vegetated ones (i.e., infiltration basins, wet basins, biofiltration swales and strips) may favor production of mosquitoes such as *Culex tarsalis, Cx. erythrothorax*, and *Aedes dorsalis* that mostly breed in habitats with vegetation (Walton and Mulla 1989). Several aspects of vegetation at the BMPs will be monitored on a bimonthly basis to assess the influence of vegetation on mosquito prevalence and production.

Measurements such as plant height, density, and percentage of vegetation cover above retained water, in addition to identifying the common species, will be documented (Southwood 1978). Plant density will be measured using 0.5 m^2 quadrats randomly placed in the BMP site. The plant density will be recorded as the average percentage of vegetation cover in the quadrat.

Monitoring Physical and Chemical Factors

Daily weather data will be collected near the location of BMPs in Los Angeles and San Diego Counties by the California Irrigation Management Information System (CIMIS), California Department of Water Resources network. The daily maximum, minimum, and average temperatures will be recorded. Precipitation information will be monitored and recorded throughout the study period. Weather patterns that have district-wide influence, especially the occurrence of storm events, may have a direct impact on mosquito production by flushing the

BMPs or providing standing water for oviposition and development of immature mosquito broods.

Water temperature has been shown to affect larval development and prevalence (Bailey and Gieke 1968, Mead and Conner 1987). When water is present, temperature, depth, pH, and concentrations of oxygen, phosphates, and nitrates will be collected on a regular basis by DHS/VBDS. Water depth will be measured by the use of a graduated stick, and then a water sample will be placed into a vial from which other water parameters will be estimated using a multi-parameter water quality meter (Hydrolab®, Ben Meadows Co. Atlanta, GA). Water quality data collected by Caltrans consultants on heavy metal (i.e., lead, zinc, and copper) concentrations and other constituents at the BMP sites will be obtained and incorporated into the database as potential factors in mosquito production.

Unusual events such as clogged drains or overflows will be documented. Such information may help provide explanations for variations in mosquito populations that otherwise would have not been associated with any of the other measured parameters.

Mosquito abatement practices

A threshold level of one larva or pupa per dip has been established to trigger application of larvicides at a BMP. This conservative control threshold limits the ability to evaluate the contribution of BMP design to mosquito production. Because of this, DHS/VBDS has adopted an abatement regime that causes the least amount of disruption to the dynamics of immature mosquitoes at the BMP sites while still providing control. Larvicides with the lowest residual effect and whose mode of action deters the emergence of pupae into adults will be preferentially used.

Liquid formulation of methoprene has a residual effect of 3-5 days and mainly disrupts the pupae-adult molting stage causing minimal effect on larval stages. The downside of using this formulation of methoprene is the potential requirement for weekly applications and measurement of larvicide applied (Ross *et al*. 1994). However, other methoprene formulations such as pellets and briquets are far less ideal in these environments because of their potential to either be covered by mud or flushed out of the BMPs during storms. Methoprene (Altosid) liquid formulation will be used at all BMPs, and will be the preferred abatement product.

However, the vector control districts may alter the larvicide used if a public health threat is eminent.

Other environmentally sound larvicides that may be used at the BMPs include *Bacillus thuringiensis israelensis* (*Bti*) and *Bacillus sphaericus*. Since these two biological agents have a longer residual effect and *B. sphaericus* has a potential to recycle (Siegel and Novak 1997), these larvicides will not be initially used.

Oil distillates are the larvicide of choice in controlling pupae. The larvicidal oils kill immature mosquitoes by suffocation (i.e., Golden Bear- GB 1111). Non-target organisms that require access to gaseous air will be impacted similarly, however GB 1111 dissipates within 36-48 hours. Its environmental impact is not well documented and it is the last choice of defense in controlling mosquito pupae and preventing adult emergence. GB 1111 is not recommended for use as a larvicide in this study, however the vector control districts may require its use.

Larvicide applications will be done by the vector control districts' certified staff; the type of larvicide, amount of water at BMP, and the quantity of larvicide per acre will be reported to DHS/VBDS. The decision to apply larvicides shall be communicated to the DHS/VBDS staff and other collaborating agencies prior to application.

Data management and analysis

DHS/VBDS will maintain a central database of all project data. Standardized data collection forms will be circulated to all agencies and contractors for data collection. Data pertaining to larval collection will be forwarded to DHS/VBDS and added to the database. A monthly data summary (hard copy or electronic file) will be disseminated to all collaborating agencies.

If the data fulfills the assumptions of parametric statistical analyses, measures of central tendency and variation in abundance will be calculated for each BMP location. If abundance data have a non-normal distribution, non-parametric measures of central tendency and variation will be calculated. For each species of mosquito, the median number of larvae/dip and percent positive dips will be compared among the different BMP designs and sites with non-parametric statistics, specifically repeated measures ANOVA based on ranked abundance, or Kruskal-Wallis test. The number of treatments by larvicides and average larvae-free period at a particular BMP site and design will be examined as ranked data. Multivariate techniques such as principal

component analysis and cluster analysis will be necessary in measuring the contributions of various parameters to mosquito abundance observed at each BMP site, especially when these data fail to meet normality tests. Data analysis will be done by DHS/VBDS in consultation with Dr. Bill Walton at the University of California, Riverside.

Study location and site selection

A total of eleven different BMP designs at thirty-four sites are currently in operation in Los Angeles and San Diego Counties. A detailed habitat description of each BMP has previously been reported (Walton 1999). Several similarity parameters between BMPs will be used to group sites for statistical purposes. Some of the similarities between sites include the BMP construction designs, the habitat type around the BMPs, and the extent of the BMP's drainage area. These parameters will be critical in assessing differences between BMP designs. Additional factors that will be considered in evaluating BMPs include historical records of mosquito collections and background population of adult mosquitoes.

To reach our primary objective of determining which BMP design is most effective in minimizing mosquito production, the BMP sites were divided into two categories: primary and secondary sites. Primary BMP sites (listed in Appendix A) will form the core for our comparative study while the secondary sites will provide the additional background information and serve as our controls. With cooperation from the Vector Control Districts and Caltrans, we recommend that the primary sites should receive limited or no modification during the study period. If abatement is necessary at any of the sites, Altosid® liquid will be the primary larvicide to be used. Since the data from these sites will be used for comparison across the BMP designs, the limiting of any factors that may introduce inconsistencies will enhance our ability to make stronger conclusions on their capacity to support mosquitoes. The BMP sites that will not be among our primary sites may be modified or treated, however, it is recommended that these interventions be communicated to us in a timely manner to be incorporated into the database.

Multi-Agency Coordination

In addition to coordinating the surveillance and control efforts of local mosquito and vector control agencies impacted by the BMP Pilot Retrofit Program, DHS/VBDS will assure uniformity in the implementation of monitoring techniques among the local agencies. Close collaboration between VBDS and the local agencies will be a priority in the implementation of the recommended abatement practices. Consultation between the local agencies and VBDS will be necessary to achieve standard larvicidal applications across the BMP sites. It is important that local agencies communicate with VBDS on any treatment applied at any of the sites.

Timeline

Year 1:   July 1, 1999 to June 30, 2000

July - September 1999

       Standardization of collection methods and database development

July 1999 - June 2000

       Data collection, archiving, and dissemination.

May - June 2000

       Preliminary data analysis.

The success of our methodology will be evaluated based on the preliminary data analysis. Various analytical tools will be used to determine observable trends within our data that significantly partition the influence of BMP design on mosquito abundance at any specific site. Any specific trend that emerges from the preliminary findings will influence the direction of the second year study.

Year 2:   July 1, 2000 to June 30, 2001

July 2000 - June 2001

       Continue data collection based on the preliminary results.

January 2001 - June 2001

       Correlation of biological, chemical, and physical parameters with mosquito production.

January 2001 - June 2001

       Data analyses and dissemination of findings to various agencies.

The strength of the conclusions from this study will depend on the amount of data collected, especially when the primary effect being tested is controlled by a multitude of density-dependent and independent factors that are equally involved in mosquito abundance without a specific controlling cluster. Given this scenario, a possibility for a third year of monitoring may be required to achieve stronger conclusions and recommendations.

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APPENDIX 1: Primary Study Sites for the BMP Retrofit Pilot Project

Biofiltration Swales

112206	I-5 Palomar Airport
112205	SR-78/Melrose
73222	N605/91

Media Filters

74204	Termination P & R
112201	Kearney Mesa
112203	I-5/La Costa P & R

Catch Basin Inserts

73216	Rosemead MS
73217	Las Flores MS
73218	Foothills MS

Extended Detention Basins

74102	S605/91
111102	I-15/SR-78
111101	I-5/SR-56

Multi-chamber Treatment Trains

74206	Via Verde
74208	Lakewood P & R
74104	Metro MS

Infiltration Trench / Bio Strip

112207	Carlsbad MS
73211	Altadena MS

Wet Basin

111104	I-5/La Costa

Infiltration Basins

73101	N605/91W
111103	I-5 La Costa